Response to Bakhoum *et al*.

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We agree with Bakhoum *et al*. [1] that whole chromosome instability involving intact chromosomes can be explained by mitotic errors. Importantly, changes in chromosome number between tumour cells are frequently accompanied by structural chromosomal alterations, often affecting the same chromosome [2,3] and multiple mechanisms linking the generation of structural and numerical aberrations have been identified [4]. Most researchers measure changes in chromosome numbers between tumour cells using centromeric fluorescence *in situ* hybridisation (FISH) [5], which cannot distinguish between aneuploidy of intact and structurally abnormal chromosomes. Limiting the definition of chromosomal instability (CIN) to whole, intact chromosomes is therefore unlikely to adequately reflect the complexity of solid tumour genomes and CIN. We therefore favour the broader definition of CIN as "losses and gains of whole chromosomes or large portions thereof" as described by the Vogelstein laboratory [6] and commonly used by others.

In their short correspondence, Bakhoum *et al*. [1] conclude that an increased frequency of lagging centric chromosomes is the most conspicuous difference between CIN+ and CIN- cells. We previously demonstrated that pre-mitotic defects, namely replication stress, underlie a high proportion of chromosome segregation errors in colorectal CIN+ cells [7]. We also observe a significantly ($p < 0.05$) higher median percentage of anaphases displaying acentrics and bridges in five CIN+ (17% and 18%, respectively) versus five CIN- (7% acentrics and 5% bridges) cell lines, confirming our original conclusion, and consistent with the established presence of acentrics in cancer cells [8,9]. The scarcity of acentrics detected in Bakhoum *et al*.'s analysis could therefore reflect differences in experimental conditions or divergence between cell line isolates. These are mitigated in our analysis by the use of positive controls (monastrol washout to induce lagging centrics and low dose aphidicolin to prevent efficient DNA replication and induce acentrics and bridges) and orthogonal methods (immunodetection of two centromere markers, and FISH using an all-centromere probe) to confirm our ability to identify lagging centrics, acentrics and bridges, and by examination of multiple cell lines of a single cancer type (colorectal) [7].

These data raise an important point about controls for CIN+ versus CIN- comparisons. Our analysis of HCT116 and DLD1 lagging centrics and anaphase bridges is concordant with Bakhoum *et al*.'s data [1,7]. However, in three additional CIN- colorectal cancer cell lines we observed low percentages of bridges (similar to DLD1 cells), indicating that bridges are significantly more frequent in CIN+ than CIN- cells within our larger, tissue-type specific cell line panel. Bakhoum *et al*. use cell lines from multiple cancer types, making comparisons more difficult, since absolute segregation error frequencies may vary between CIN+ breast, cervical and colorectal cancers.

One conclusion of our study [7] was that replication stress can lead to deviation of centromere numbers determined by centromeric FISH. Importantly we demonstrated this deviation in cell clones grown for multiple generations following induction of replication stress by depletion of PIGN, ZNF516 or MEX3C, demonstrating ongoing change in centromere number as observed in CIN+ cells [1,6]. Our findings that a high proportion of chromosome segregation errors in CIN+ colorectal cancer cells are pre-mitotic in origin, combined with multiple additional experiments demonstrating elevated replication stress in CIN+ compared to CIN- colorectal cancer cell lines, led us to conclude that replication stress-induced chromosome missegregation is an important contributor to CIN in colorectal cancer [7]. In our opinion, data presented by Bakhoum *et al*. in this issue of *Current Biology* [1] and previously, demonstrating reduced chromosome missegregation and deviation of centromere numbers upon suppression of mitotic defects, do not contradict the conclusions of our recent study [7], nor do they minimize the importance of DNA replication stress as a contributor to CIN in colorectal and other cancers [10]. In contrast, our observations, together with the data of Bakhoum *et al*., suggest multiple mechanisms likely contribute to CIN both within and between tumour types [4].

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